REMARKS

Status of the Claims

Claims 1-15 and claims 24-36 were pending in the application.

Claims 24-26 were withdrawn.

Per the Office Action Summary, claims 1-15 and 27-36 were rejected. In the Detailed Action, however, no rejections have been set forth for claims 5, 7, 10, 12, 15 and 32.

Claims 1-6, 11, 29, and 31-36 were objected.

By way of this amendment, claims 1-4, 13, 14 and 27 have been amended, claims 24-26 and 30 have been canceled and new claim 37 has been added.

Upon entry of this amendment, claims 1-15, 22-29 and 31-37 will be pending.

Summary of the Amendment

Claims 1-4 and 27 were amended to correct obvious typographical errors.

Claim 13 has been amended to define embodiments of the invention. Support for claim 13 as amended is found throughout the specification and claim 14 as filed.

Claim 14 has been amended to more clearly define embodiments of the invention. Support for claim 14 as amended is found throughout the specification and claims as filed.

New claim 37 has been added to more specifically and clearly refer to embodiments of the invention. Support for new claim 37 is found throughout the specification, such as on page 5, and claim 21 as filed.

No new matter has been added. Likewise, the amendment raises no new issues.

Claim Objections

Claims 1 and 33, claims 2 and 34, claims 3 and 35, claims 5 and 32, claims 6 and 29 and claims 11 and 31 have been objected to as being improper under 37 CFR 1.75(c). The Office asserts that the dependent claims fail to further limit the subject matter of the previous claim. Applicants respectfully note that the objection to the claims may have been improperly applied.

Applicants respectfully point out that the subject matter of the claims set forth in pairs on page 2 of the Official Action is different. For example, claim 1 refers to **a biologically pure bacterial culture of at least one mutant strain** of *P. fluorescens,* whereas claim 33 refers to **a pure mutant strain** of *P. fluorescens*. As set forth in the claim, the biologically pure bacterial culture defined in claim 1 **comprises "at least one mutant strain**," i.e., it can comprise **more then one** strain. Claim 33 is defined as "a pure mutant strain," i.e. **a single strain**. Although both claims set forth that the *P. fluorescens* produces at least 10 g alginate per liter medium, the culture of claim 1 may contain two or more different strains of *P. fluorescens* while claim 33 defines a pure mutant strain. The scope of each claim is clearly different from that of the other. Likewise, the scope of each claim of claims 2 and 34, of claims 3 and 35, of claims 4 and 36, of claims 5 and 32, of claims 6 and 29, and of claims 11 and 31 is different from that of the other claim of the respective pair.

Claims 1-4 and 27 were objected to for apparent grammatical errors. Applicants have amended claims 1-4 and 27 in order to correct the grammatical errors.

Applicants respectfully request that the objection to the clams be withdrawn.

Claim Rejections Under 35 U.S.C. §112, Second Paragraph

Claims 13, 14, and 30 stand rejected under 35 U.S.C. §112, second paragraph, for allegedly failing to comply with and particularly point out and distinctly claim the subject matter of the current invention.

Claim 13 is rejected based upon the use of the term "effector gene." Applicants respectfully note that one of ordinary skill in the art at the time of filing would know the meaning of the term "effector genes" as used in the claims and specification. Nevertheless, the term has been deleted and the rejection is moot.

Claim 14 is rejected based upon the use of the terms "Pm promoter" and XylS gene." It is asserted in the Official Action that it is unclear what Pm means and what is the source of the XylS gene. Applicants respectfully note that one of ordinary skill in the art at the time of filing would know the meaning of the term "Pm promoter" as used in the claims and specification.

Likewise, one of ordinary skill in the art at the time of filing would recognize the XylS gene and know the sources from which it could be derived and used. No basis has been offered to raise doubt as to the clarity and meaning of these terms.

Claim 30 is rejected as being indefinite. Claim 30 has been canceled and the rejection is moot.

Applicants respectfully request the rejection of claims 13 and 14 under 35 U.S.C. §112, first paragraph be withdrawn.

Claim Rejections Under 35 U.S.C. §112, First Paragraph

Written Description

Claims 1-4, 6, 8, 9, 11, 13, 14, 27-31, and 33-36 stand rejected under 35 U.S.C. §112, first paragraph, for allegedly containing subject matter which was not described in the specification in such a way to reasonably convey to one skilled in the art that the inventors, at the time of the application was filed, has possession of the claimed invention. The Office specifically asserts that:

> the disclosed strain do not adequately represent all of the structure and/or function of all strain encompassed by the instant claims. The Applicant does not teach how all these diverse strain will be modified to have recited function.

(Office Action, page 5). Applicants respectfully disagree and request that the rejection be withdrawn.

Applicants respectfully urge that the description of the claimed invention including the examples as set forth in the specification demonstrates that Applicants were in possession of the claimed invention at the time the application was filed. The specification includes description and data generated using embodiments of the invention. Moreover, the description was complete in its description of the invention including distinguishing identifying characteristics sufficient to show that the applicant was in possession of the claimed invention.

An adequate written description of the claimed invention was present when the application is filed. The reasoning offered in the Official Action as to why persons skilled in the

art would not recognize in the disclosure a description of the invention defined by the claims is insufficient to support the rejection. The claimed invention as a whole was adequately described; the claims reflect the essential and critical features of the invention which are set forth in both the specification and claims as filed. The claims refer to *P. fluorescens* with specific characteristics. Those skilled in the art would recognize that the identity of *P. fluorescens* as a structural limitation which is coupled with certain functional characteristics set forth in the claims. The claims are structurally limited. In addition to the examples, one skilled in the art can immediately envisage the claimed invention from the disclosure.

It is well settled that the Office has the initial burden of presenting evidence or reasons why a person skilled in the art would not recognize that the written description of the invention provides support for the claims. There is a strong presumption that an adequate written description of the claimed invention is present in the specification as filed. The reasons provided by the Office as to why a person skilled in the art would not recognize that the written description of the invention provides support for the claims do not overcome presumption that an adequate written description of the claimed invention is present in the specification as filed.

Claims 1 and 33 are the independent claims rejected as failing to comply with the written description requirement. Claim 1 recites:

> A biologically pure bacterial culture of at least one mutant strain of *P. fluorescens,* wherein said strain produces at least 10 g alginate per liter medium.

Claim 33 recites:

> A pure mutant strain of *P. fluorescens* which produces at least 10 g alginate per liter medium.

Each claim has sufficient structure and function to make clear the scope and meaning of the claim. Each claim refers to *P. fluorescens* which produces at least 10 g alginate per liter medium. Those skilled in the art can readily recognize the structural and functional limitations of the claims as well as the fact that Applicants were in possession of the invention at the time the application was filed. Similarly, the scope and meaning of the dependent claims rejected as failing to comply with the written description requirement is also clear. Those skilled in the art

can readily recognize the structural and functional limitations of such claims as well as the fact that Applicants were in possession of the invention at the time the application was filed.

Applicant provides adequate support to demonstrate possession of the claimed invention the time the application was filed. The examples in the specification clearly demonstrate that Applicants were in possession of the claimed invention. In addition, the claimed invention is clearly described and the specification and claims as filed to permit a person skilled in the art to clearly recognize that applicants had possession of the claimed invention at the time the application was filed.

The disclosure supporting the claimed genus satisfies the written description requirement. A representative number of species are disclosed in the examples and description of the invention. The specification contains sufficient disclosure to show the applicant was in possession of the claimed genus. A "representative number of species" means that the species which are adequately described are representative of the entire genus. Thus, when there is substantial variation within the genus, one must describe a sufficient variety of species to reflect the variation within the genus.

The specification discloses nine species of the claimed invention which comprise alterations of one of five different gene targets or introduction of a heterologous gene construct. Such disclosure is "representative number" of the **claimed invention.** One skill in the art would recognize that the applicants were in possession of the necessary common attributes or features of the elements possessed by the members of the genus of the **claimed invention** in view of the species disclosed.

It is not required that Applicants disclose every modification that could yield the recited function. Applicants have disclosed five gene targets in which mutations have produced strains according to the invention. Applicants have additionally strains according to the invention produced by the addition of genetic material. The disclosure of these diverse species of *P. fluorescens* which share the common functional attributes is sufficient to evidence that Applicants were in possession of the claimed invention at the time the application was filed.

The claims are in compliance with the requirements of the first paragraph of 35 U.S.C. §112, first paragraph. Applicants respectfully request that the rejection based upon 35 U.S.C. §112, first paragraph be withdrawn.

Enablement

Claims 1-4, 6, 8, 9, 11, 13, 14, 27-31, and 33-36 stand rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing the enablement requirement. The Office asserts that the specification does not provide sufficient guidance to enable one of ordinary skill in the art to make and use the claimed invention in a manner reasonably correlated with the scope of the claims. The Office asserts that the claims are not enabled because the specification:

> Does not establish (A) regions of DNA structure of algG gene which should be modified to control alginate synthesis activity and/or how to control by any means the algG gene or any gene involved in alginate synthesis to obtain desired function without effecting other genes that involve the alginate synthesis; (B) the general tolerance of algG gene or other alginate biosynthetic genes to modification and extent of such tolerance towards controlling the gene with any means; (C) a rational and predictable scheme for modifying any algG residues or residues of other genes with an expectation of obtaining the desired biological function (D) the specification provides insufficient guidance as to which of the essentially infinite possible choices is likely to be successful.

(Office action, Pages 7-8).

Applicants note that it is well established that the Office has the initial burden of establishing that a claimed invention does not meet the enablement requirement. The description of the invention is presumed to be enabled and, in order to sustain an enablement rejection under the first paragraph of 35 U.S.C. §112, the Examiner must establish doubt in the objective truth of Applicant's assertion that the claimed invention is enabled using reasoning and evidence of those

skilled in the art. See, e.g. *In re Marzocchi*, 439 F.2d 220, 224, 169 USPQ 367, 370 (CCPA 1971). See also M.P.E.P. § 2163.

The Office has failed to set forth any evidence to support the rejection. Rather, the issues raised on page 9 of the Official Action are provided as conclusion of non-enablement based upon the assertion that because the claims encompass strains "having mutation of any number of alginate biosynthetic pathway genes" and there are many ways to produce a strain "having mutation of one or more alginate biosynthetic pathway genes" the claims are not enabled. Applicants urge that the specification provides an enabling disclosure. Moreover, the Office has not established that the claimed invention does not meet the enablement requirement. Failing to do so, the burden is not properly shifted to Applicants.

Applicants respectfully urge that the evidence in the specification supports the conclusion that one skilled in the art would accept Applicant's assertion that the claims are enabled by the specification. In the absence of any evidence and reasoning in support of the rejection, Applicants are not required to put forth any evidence.

The specification describes how to make the claimed invention. Claims 1-4, 13-14 and 33-36 relate to strains in which the functional limitation relate to alginate production levels. Claims 6, 8-9 and 27-31 further relate to characteristics of the alginate that is produced (molecular size, acetyl content, G-content).

The specification enables one skilled in the art to make strains that can produce alginate at the production levels set forth in the claims. Using the methods described in the specification, a person skilled in the art would be able to obtain strains that can produce alginate at the production levels set forth in the claims without undue experimentation. The specification describes two ways of producing strains that can produce alginate at the production levels set forth in the claims. The first such method is by random mutagenesis and screening as described in Example 1. The other method is by controlling the synthesis of the alginate biosynthetic genes. These are the two fundamentally most important ways to increase the alginate production in any strain of *Pseudomonas fluorescens*.

Random mutagenesis as described in the specification can be used by those skilled in the art would be able to obtain strains that can produce alginate at the production levels set forth in the claims without undue experimentation. As disclosed in the specification, inactivation or reduced activity of negative regulators or competing metabolic pathways results in the increase in alginate production. Mutants can be produced and screened routinely. The specification discloses how to perform these methods in a way that allows for screening of hundred of thousands mutants using equipment found in any microbiology laboratory. The screening procedure exploits the ability of strains that produce alginate as set forth in the claims to grow on plates containing far more carbenicillin than what is tolerated by the strains that do not produce alginate. In this way ten thousand mutants, or even more, may be spread on a single agar plate and only the desired mutants will grow. As known to persons skilled in the art, when such a mutant is found it can be analyzed to identify the mutation and the same mutation or other mutations in the cell gene can be introduced by site specific mutation. The specification describes several examples of strains according to the invention.

Control of expression of the alginate biosynthetic genes is also described in the specification sufficiently such that it can be used by those skilled in the art to obtain strains that can produce alginate at the production levels set forth in the claims without undue experimentation. As disclosed in the specification, these genes are found in one operon and one single gene (*alg*C). They are well conserved between species and can be cloned by PCR from any strain containing these genes. The specification discloses that overexpression of all the genes in the alginate biosynthetic operon, yields a high level of alginate production. In the example the *Pm*-promoter of the benzoate meta-cleavage pathway from the Tol-plasmid of *Pseudomonas putida* and its cognate regulatory protein XylS are used.

The specification enables one skilled in the art to make strains that can produce alginate at the production levels set forth in the claims.

Likewise, the specification enables one skilled in the art to make strains that can produce alginate at the production levels set forth in the claims in which the characteristics of the alginate that is produced (molecular size, acetyl content, G-content) is as set forth in the various claims.

The specification describes several alterations in *Alg*L, *Alg*G and *Alg*lJF. These mutations produce more specific types of alginate.

The specification provides a detailed description of the several alginate biosynthetic genes and how to regulate one gene independently of the others; namely to make an in-frame-deletion of most of the gene and then introduce a transposon containing the gene controlled by an inducible promoter. In Example 6, this is performed with *Alg*G. It is further shown how mutations and site specific recombination can be performed on genes within the alginate biosynthetic operon. The disclosure in the specification is sufficient such one skilled in the art can practice the invention without undue experimentation.

The specification discloses that by deleting/inactivating *Alg*F no acetyl groups are linked to the alginate, while deleting/inactivating *Alg*J few acetyl groups are linked to the alginate. Using these teachings, one skilled in the art could achieve these results without undue experimentation. The principle is shown: alginate with a few or no acetyl groups can be obtained by inactivating *Alg*F or *Alg*J respectively. To inactivate a gene by deletions or multiple substitutions is routine. These proteins are shown to be not needed for the alginate biosynthesis to function normally. Deletion of a necessary part of the protein, as described in the specification, is the preferred way of inactivating a gene but those skilled in the art could readily achieve this result routinely using other strategies and techniques.

The specification discloses strains that produce alginate with no G-residues. Such strains can be found by randomly mutating alginate strains of the invention and screening as described in Example 3. This is the most preferred way to obtain such mutants. The specification also discloses two other ways to obtain such strains. Strains in which *Alg*G is mutated to produce no epimerase activity of have *Alg*G with reduced activity. A library can be screened to identify randomly mutagenized *Alg*G -genes as described in Example 6 as well.

The specification discloses strains that produce alginate with a defined number of G-residues. The specification discloses two ways to obtain such strains. These methods are set forth in Examples 6 and 7. The method in Example 6 does not, as described above, rely on any prior information except the primary sequence of the gene. The equipment needed is a plate

reader and an incubator. Bacteria can be grown in 96 well-plates put into ordinary plastic boxes and incubated in ordinary incubators. Strains may be identified by sequencing PCR-amplified algG. Proteins with reduced activity may be made by using site-specific mutagenesis.

The specification provides sufficient disclosure to enable one skilled in the art to practice the claims invention without undue experiments. The claims are enabled. In view of the foregoing discussion, Applicants respectfully request that the rejection of the claims under 35 U.S.C. §112, first paragraph, be withdrawn.

Claim Rejections Under 35 U.S.C. § 102

Claims 1-4 and 33-36 have been rejected under 35 U.S.C. §102(e) as being anticipated by Huisman *et al.* It is asserted that Huisman discloses *Pseudomonas fluorescens* that produces 40% dry cell weight of products that include alginates and that the fermentation process disclosed in Huisman produces 50g/l, presumably suggesting that Huisman discloses *Pseudomonas fluorescens* that produces 20 grams of alginate per liter of cells. Applicants respectfully disagree with the characterization in the Official Action of the facts disclosed by Huisman and respectfully urge that Huisman does not anticipate the claimed invention.

There is not disclosure in Huisman that strains of *Pseudomonas fluorescens* produce 80g/l of alginates. Huisman does not disclose that products produced by any strains of *Pseudomonas fluorescens* are all or even mostly alginates.

Claim 1 in Huisman refers to bacterial strains useful to produce products including polysaccharides which comprise a genetically modified nuclease gene. Claims 5 and 7, which are each dependent on claim 1, refer to products such as alginates, and bacteria such *Pseudomonas fluorescens* as further limitations on claim 1. Claims 2 and 3 are also cited in the Official Action. Claim 2, which is dependent on claim 2, refers to strains that grow to a density of 50 g/l. Claim 3, which is dependent on claim 2, refers to strains of claim 2 which produce polyhydroxyalkanoates to levels of at least 40% of its dry cell weight. Alginates are not polyhydroxyalkanoates, and polyhydroxyalkanoates are not alginates. Claim 3 does not disclose strains of any bacteria that grow to a density of 50 g/l and produce alginate, or any other product

other than polyhydroxyalkanoates, to levels of at least 40% of its dry cell weight. The claims asserted by the Office as anticipating the claims in the instant invention do not disclose the instantly claimed invention. Further, nowhere in the disclosure of Huisman is there any suggestion of strains of *Pseudomonas fluorescens* or any other bacteria which produce alginates at the level disclosed in claim 3 of Huisman for producing polyhydroxyalkanoates

Huisman does not disclose strains of *Pseudomonas fluorescens* which produce up to 80g/l of alginates. Huisman does not disclose strains of *Pseudomonas fluorescens* which produce up to 10g/l of alginates. There is no reasonable interpretation of Huisman which supports the conclusion that is discloses strains of *Pseudomonas fluorescens* which produce up to 10g/l of alginates.

Huisman teaches a solution to the problem commonly encountered when harvesting products from bacteria. When bacterial cells are lysed during the process to isolate products from the cells, the nucleic acid of the bacteria causes an increase in viscosity of the lysate which makes harvesting products more difficult. Nucleases may be added to lysates when harvesting products from bacteria in order to prevent the increase in viscosity. Huisman provides an alternative solution by providing bacterium which produces nuclease to degrade nucleic acids in order to enhance product recovery.

As for the bacteria and products, Huisman refers to *Pseudomonas fluorescens* as a bacteria used in productions and refers to alginate as one of several products but Huisman does not refer to *Pseudomonas fluorescens* which produce up to 10g/l of alginates.

In order for a reference to anticipate a claim, every element of the claim must be found in the reference. Huisman does not anticipate the claims because Huisman does not refer to *Pseudomonas fluorescens* which produce up to 10g/l of alginates. The disclosure in Huisman of strains of bacteria which produce up to 50g/l of cells of which 40% dry cell weight is polyhydroxyalkanoates is not a disclosure of *Pseudomonas fluorescens* which produce up to 10g/l of alginates. Huisman cannot be properly read to conclude that it discloses *Pseudomonas fluorescens* which produce up to 10g/l of alginates. Huisman does not anticipate the claims.

Applicants respectfully request that the rejection of claims 1-4 and 33-36 under 35 U.S.C. §102(e) as being anticipated by Huisman be withdrawn.

Conclusion

For the foregoing reasons, Applicants respectfully request that the claims be allowed at this time. A notice of allowance is earnestly solicited.

The Commissioner is hereby authorized to charge any deficiencies of fees and credit of any overpayments to Deposit Account No. 50-0436.

Respectfully Submitted,

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